EXTRACTION METHOD OF ACTIVE MOLECULAR STRUCTURES FROM NATURAL RESINS AND/OR ESSENTIAL OILS

The present invention relates to an extraction method of active molecular structures from natural resins and/or essential oils.

In particular, it relates to a method for the extraction of terpenes and/or terpenoids from natural resins and/or essential oils.

Natural resins, such as myrrh, incense, dacryodes, propolis, are substances which contain a high quantity of terpenes and terpenoids.

The types of terpenes contained in these natural resins are mainly: sesquiterpenes (C_{15} structure), diterpenes (C_{20} structure) and triterpenes (C_{30} structure).

In the above resins, together with these active substances, there are also sugars starches and Rosinic resins (rosins) and the active substances are imprisoned inside these polymeric components.

In particular, DSC tests effected with a Perkin-

Elmer Pyris apparatus on the above natural resins, incense, myrrh, dacryodes and propolis, have shown that these resins have a polymeric-type structure. The polymeric structure is characterized by a Tg equal to 80°C for incense, a Tg equal to 60°C for myrrh and a Tg equal to 85°C for dacryodes.

The DSC plots show that incense has a structure of the amorphous type, with a melting point of 130°C, myrrh has a semi-crystalline structure with a melting point of 140°C, dacryodes has a semi-crystalline structure with a melting point of 250°C.

Among the three resins examined, only dacryodes has the capacity of crystallizing at a temperature of 180°C. The dacryodes resin consequently behaves like a polymer with phase transition, i.e. an amorphous polymer which crystallizes when subjected to heat.

The traditional extraction method of the active substances contained in these resins is extraction in a vapour stream. The extraction system with supercritical CO_2 has also been recently introduced and used for these substances. The extraction method with supercritical CO_2 allows the extraction of a higher number of active substances, with a greater yield, but creates problems of reaction between the substances themselves. For this reason, the most widely used method is still vapour stream

extraction.

Through vapour stream extraction, it is possible to extract the following mixtures of compounds, also called essential oils, contained in incense, myrrh, dacryodes and in propolis:

A) INCENSE: the essential oils extracted in a vapour stream from incense prove to consist of:

- ALPHA PINENE monoterpene;
- ALPHA THUJENE monoterpene;
- LIMONENE monoterpene;
- P-CIMENE monoterpene;
- BETA-MYRCENE monoterpene;
- GURJUNEN sesquiterpene;
- OLIBANOL ketonic alcohol

with small quantities of other substances. The average extraction yield is equal to 1.70%-2% by weight.

B) MYRRH: the essential oils of myrrh extracted in a vapour stream prove to consist of:

- FURANEUDESMA-ALPHA-1,3-DIENE sesquiterpene;
- CURZERENE sesquiterpene;
- LINDESTRENE sesquiterpene;
- ELEMENE sesquiterpene;
- COPAENE terpenoid;
- 1-CADINOL sesquiterpenoid;
- GERMACRENE sesquiterpene

with small quantities of other substances. The average extraction yield is equal to 2.80%-3% by weight.

C) DACRYODES: extraction in a vapour stream did not allow an essential oil of the commercial type to be obtained.

D) PROPOLIS:

- 2-methyl-3-butenol
- 3-penten-2-ol
- 3-methyl-buten-1-ol
- toluene
- 2-methyl-buten-1-ol
- ethyl-benzene
- 1,3,5,7-cyclooctatetraene
- 2-buten-1-ol-3-methyl-acetate
- benzaldehyde
- beta-myrcene
- octanal

Beta-myrcene is the only terpene extracted, with a total extraction of 10 compounds and an average extraction yield of 6%.

Scientific literature (1998) indicates the following compositions for incense and myrrh:

- Incense: 50-70% BOSWELLIC ACIDS;
  4-8% PINENE-PHELLANDRENE-TERPENE ALCOHOLS
  20% RUBBER SUBSTANCES;
  6-8% POLYSACCHARIDES.

The active substances therefore form 73-75% by weight, whereas the inert substances form 28-30% by weight with respect to the total weight of the resin.

From what is specified above, it can be deduced that the major components of incense are Bosellic acids which are triterpenes.

- Myrrh: 30% DELTA ELEMENE;
  10% ALPHA COPAENE;
  12% FURANEUDESMA-1,3-DIENE;
  4% LINDESTRENE;
  12% ISOFURANGERMACRENE AND CURZERENONE;
  20% TRITERPENES;
  12% STARCHES AND VEGETABLE PROTEINS.

The active substances therefore form 40% by weight, whereas the inert substances form 60% by weight with respect to the total weight of the resin.

From what is specified above, it can be deduced that triterpenes represent a high quantity in myrrh, equal to 20% by weight of the resin.

From a simple comparison with the data indicated above relating to products extracted in a vapour stream from incense and myrrh, it can be observed that the triterpenes have not been extracted in incense, whereas the triterpenes and part of the sesquiterpenes have not been extracted in myrrh.

The objective of the present invention is therefore to find a new extraction method which allows the disadvantages of the known art indicated above to be overcome.

The object of the present invention consequently relates to an extraction method of terpenes and/or terpenoids from natural resins, such as myrrh, incense, dacryodes, dammar, propolis and/or essential oils, by means of extraction with polar and/or semi-polar solvents in the presence of a rotating magnetic field.

The polar and/or semi-polar solvent is preferably selected from ethanol and ethanol/ethyl ethanoate mixtures.

Even more preferably, the polar and/or semi-polar solvent is ethanol, in particular pharmaceutical ethanol.

Pharmaceutical ethanol refers to extremely pure neutral ethanol 96% vol. with a maximum content of contaminants equal to 0.058 mg/l.

The solvent is present in a quantity which varies from 10 to 90%.

The rotating magnetic field has an intensity varying from 600 to 3000 Gauss, preferably from 1500 to 3000 Gauss.

The extraction is effected at a temperature which ranges from 30°C to 75°C, preferably from 35°C to 60°C and is carried out for a time ranging from 15 to 120 min-

utes, preferably from 30 to 60 minutes.

An extraction method by means of extraction with ethanol at a temperature ranging from 35°C to 60°C, for a time varying from 30 to 60 minutes, with a rotating magnetic field having an intensity ranging from 1500 to 3000 Gauss, is particularly preferred.

Very surprisingly in fact, the method according to the present invention allows all the active substances present in incense, myrrh, dacryodes and propolis, to be extracted, with the removal of the rubbery part, starches, sugars and vegetable proteins.

It allows the extraction of compounds having molecular weights higher than 600 dalton.

Similarly, the method according to the present invention allows essential oils to be obtained, in which terpenes and/or terpenoids, flavonoids and steroids are present as free molecules. The essential oils, in fact, obtained by extraction in a vapour stream or with supercritical CO_2 are not aerodispersible or at least only to a minimum degree.

A fundamental advantage of the extraction method according to the present invention is that it allows solutions to be obtained at different concentrations. By varying the ratio between time/temperature/magnetic field intensity, it is in fact possible to cover the whole

range of extractions of all types of resins and/or essential oils partially soluble in ethanol and ethyl ethanoate and mixtures of these two solvents in any proportion.

A further object of the present invention also relates to the alcohol and/or hydro-alcohol solutions obtained with the extraction method according to the present invention, containing free molecular structures of sesquiterpenes, terpenes, triterpenes, terpenoids, flavonoids and/or steroids.

In particular, these solutions are dispersible in air within the temperature range of 40°C to 90°C, preferably with the use of thermo-emanators or electro-emanators. They are preferably perfectly dispersible in air within the temperature range of 80 to 90°C.

An object of the present invention also relates to the solutions obtained with the extraction method according to the present invention mixed with each other, mixed with all types of essential oil, in any proportion, and/or mixed with water, up to a maximum of 25% of distilled water, whatever the proportion of the solutions with each other may be.

Solutions are preferred, obtained with the extraction method according to the present invention, in a mix, comprising fractions of terpenes and/or terpenoids ex-

tracted from incense in a percentage ranging from 15 to 65% by weight, fractions of terpenes and/or terpenoids extracted from myrrh, in a percentage of between 15 and 60% by weight.

Solutions are also preferred obtained by means of the extraction method according to the present invention, which includes fractions of terpenes and/or terpenoids extracted from incense, myrrh and propolis in a mix.

In particular, solutions are also preferred containing Hyssopus officinalis decumbens or Hyssopus officinalis aristatus, green tangerine, fractions of terpenes and/or terpenoids extracted from myrrh and fractions of terpenes and/or terpenoids extracted from incense, in particular proportions equal to about 1:0.3:5:5.

The characteristics and advantages of the extraction method according to the present invention will be more evident from the following detailed and illustrative description, referring to the following examples.

EXAMPLE 1.

A general description follows of the various phases.

Grinding of the resin:

a) grinding of the resins to a particle size of 20 microns using a mill with cross blades. The grinding chamber of the mill is maintained at a temperature

of about 15°C to prevent the formation of large-dimensioned agglomerations during the grinding process;

b) the ground product is stored in air-tight containers;

c) the grinding is effected under a suction hood to prevent the depositing of fine powder which, in the case of incense, could cause flammability phenomena in air (flashing).

Preparation of the solution to be subjected to the extraction process:

The resins thus ground are mixed with pharmaceutical ethanol in a ratio of 10:1 (ten parts of ethanol per one part of resin). The resins are then left to soak in the ethanol for 15 minutes in sealed glass container.

Extraction:

The ethanol-resin solution is placed in a Pyrex glass flask with a flat bottom and positioned on a heatable magnetic stirrer.

The flask is closed with a sealing glass plug and parafilm.

A magnetic anchor coated with Teflon is placed in the solution. The temperature is regulated at 50°C and the stirring rate is regulated at the maximum value (1,500 rpm).

These operating conditions are maintained for 60 minutes.

The temperature is then brought to 23°C and the stirring rate is decreased (500 rpm).

These conditions are maintained for 15 minutes.

The solutions are left to rest for 30 minutes at room temperature.

The magnetic field is then induced by the rotation of a permanent magnet having a field intensity equal to 3000 Gauss.

Filtration:

The solutions obtained are filtered using filter paper for qualitative analysis with a filtering capacity of 5 microns, in order to prevent the passage of any particle remaining in solid form.

The solutions are left to rest for 15 minutes and subsequently filtered again using the same type of filter.

Results:

Limpid solutions with an amber-yellow colour are obtained, absolutely free of impurities and particulate.

Analysis of the solutions obtained

Analysis of the alcohol solutions thus obtained was effected by means of Mass gas-chromatography (GC/MS and GC/FID) using a Hewlett-Packard HP 6890 instrument with

Supelco SBP-5 and Supelco-wax 10 columns.

Solution prepared starting from incense.

The extraction method according to the present invention starting from incense as natural resin produced a solution in which the following compounds were identified:

alpha pinene,

alpha thujene,

(+) limonene,

p-cymene,

beta-myrcene,

2-propyl-5-methylnaphthoquinone,

furandiene,

cembrene A (cembra-3,7,11,15-tetraone),

1-isopropenyl-3-propenylcyclopentane,

germacrene A,

2-N-butyl-8-N-hexyl-1,2,3,4-tetrahydronaphthalene,

verticellol (Verticilia (20) 7,11-triene),

aciphyllylic alcohol,

pentacyclododecane,

globulol 1H-cyclopropen(e)azulene,

17-alpha-methyl-5-alpha-androstane,

1,5-dimethoxy-2,6-bis (prop-2'-enyl)anthraquinone,

E-Ocimenone 2,6-dimethyloctane,

Trunculin-F methyl ester,

4,7-methanoisobenzofuran-1-ol 1,2,3°4,7,7° hexahydro

Eicosane,

4,8-decadienoic acid 2-acetyl 2,5,9 trimethyl

Longicyclene,

7-hydroxyoctanoic acid 2TMS,

Aristolone 2H-cyclopropene-naphthalen-2-one,

beta-boswellic acid,

acetyl beta-boswellic acid,

11-keto-beta-boswellic acid,

acetyl 11-keto-boswellic acid,

trans-octahydro-5,5-dimethyl-8a-tosyloxymethyl 2

11-H indene (1.2-B) quinoxalin-11-11H-indenol

3A-hydroxyolean-12-en-24 boswellic acid,

Total active products present: 32.

Extraction yield: 56% out of a total of 72-75% of active substances.

Solution prepared starting from myrrh.

The extraction method according to the present invention starting from myrrh as natural resin produced a solution in which the following compounds were identified:

alpha-terpinene para-mentha 1,3-diene,

beta-bourbonene,

delta-elemene,

trans-caryophyllene,

6-ethenyl-4,5,6,7-tetrahydro 5-benzofuranacetic acid,

1-cyclohexene-1-carbonaldehyde,

gamma-cardinene,

germacrene B

2-ter-butyl-1,4-naphthoquinone,

(+)hepi-bicyclosesquiphellandrene,

furaneudesma-1,3-alpha diene,

Ledene alcohol,

alpha-longipinene,

alpha-copaene,

1-ethyl-1,2,3,4-tetrahydro 2-naphthalenemethanol,

benzylthio-6-methylluracin 2 (1H) pyrimidinone,

1,3-dimethyl-5-azulenecarbaldehyde,

dibenzofuranol-1,4-dicarbaldehyde 1,3-naphthalenate,

germacrene A,

2-isopropyl-4,7-dimethyl-1-naphthol,

bicyclogermacrene,

lindestrene,

4-(phenylmethyl)phenol,

amino-3(1)imidazo (4,5H)quinoline,

methyl 5-hydroxy-3-oxo-5-phenylpentanoate,

2-cyclohexyl-1-(1H-imidazol-2-yl)ethanone

beta-amirine,

(Urs-12-ene,3-methoxy,3 beta) beta-commiphoric acid.

Total active products present: 28.

Extraction yield: 35% out of a total of 40% of active substances.

Solution prepared starting from dacryodes.

The extraction method according to the present invention starting from dacryodes as natural resin produced a solution in which the following compounds were identified:

delta-elemene,

alpha-copaene,

beta-bourbonene,

beta-elemene,

trans-caryophillene,

alpha-amorphene,

beta-silenene,

germacrene B,

caryophyllene oxide,

epicurzerenone,

1,4-dimethyl-7-(1-methylethyl)-azulen-2-ol,

alpha-cadinol,

furandiene,

2-isopropylquinoline-beta-D6,

vulgarol A,

phenoxazone,

seychelene,

1-naphthalenemethanol alpha, alpha-dimethyl-5,

2,3 dihydroindole-4-ol-2-one,

2-pentyl-1H-quinolin-4-one,

1,1,4,6,7-pentamethyl-2,3-dihydroindene,

dibenzothiophene,

germacrene A,

2-isopropyl-4,7-dimethyl-1-naphthol,

4-quinolinol, 2 pentyl-N-oxide,

methyl-3-methyl-2-thiophenecarboxylate,

2(5H)furanone, 3 butyryl-5-hexyl-4-hydroxy-5-methyl duvantriendiol,

1,2-epoxynonane,

(+) alpha-ciperone,

6,6-dimethylundecane 1,11dyl bis (2-hydroxy-5-(2-carot)-1,4-dienaldehyde.

Total active products present: 32

Extraction yield: 13%.

It is not possible to calculate a ratio between the extracted quantities and the total quantity of active substances, as no composition for this resin has been cited in literature.

The triterpene compounds were identified not only with the GC/MS system but also by means of gas chromatograph in liquid phase (HPLC) in UV light in a wave length of 210 nm.

EXAMPLE 2

A general description of the different phases is provided.

Grinding of the resin

a) Propolis is in the physical form of very consistent waxy cakes. Grinding is effected in order to obtain an average diameter of the particles ranging from 80 to 100 microns, making use of a crossed blade mill. The grinding chamber of the mill is maintained at a temperature of 15-18°C to prevent the formation of large dimensional clumps during the grinding.

b) The ground product is stored in a hermetically sealed container.

c) Grinding is effected under a fume hood in order to prevent deposition of fine powders which can give rise to flashing phenomena.

Preparation of the solution to be subjected to the extraction process:

The propolis thus ground is mixed with pharmaceutical ethanol in the ratio 7:1 (seven parts of ethanol per one part of resin). The resin is left to soak in ethanol for 30 minutes in a sealed glass container.

Extraction:

The ethanol-propolis solution is poured into a Pyrex glass flask with a flat bottom and placed on a heated magnetic stirrer. The flask is closed by means of a

sealing glass plug and parafilm. A magnetic, Teflon-lined anchor is placed in the solution. The temperature is regulated at 40°C and the stirring rate at the maximum value(1,500 rpm).

These operating conditions are maintained for 45 minutes. The temperature is then lowered to 23°C and the stirring rate to 500 rpm. These conditions are maintained for 30 minutes at room temperature. The magnetic field is then induced by the rotation of a permanent magnet with an intensity of 3,000 Gauss.

Filtration

The solution obtained is filtered by means of a paper filter for qualitative analysis having a filtering capacity of 5 microns, in order to prevent the passage of any small particles in solid form.

The solution is left to rest for 15 minutes and then filtered again.

The resulting filtrate is left to rest for a further 15 minutes and is then filtered again, still using the same type of filter.

Results:

A limpid brown-coloured solution is obtained, absolutely free of impurities and particulate.

Solution prepared starting from propolis.

The extraction method according to the present inven-

tion, starting from propolis as natural resin, gave a solution in which the following compounds were identified by means of GC/MS and GC/FID analysis:

guaiole;

10-epi-gamma-eudesmole;

beta-eudesmole;

2-propenoic acid;

isopropyl tetradecanoate;

alpha-eudesmole;

8-beta-H-cedran-8-ole;

ethyl ester of heptanoic acid;

n-pentacosane;

butyl ester of hexadecanoic acid;

n-tetracosane;

butyl stearate;

androstan-4-en-3-one;

hexacosane;

benzo[c]naphthol[2,1-p]crysene;

dihydroxymethylcalcone;

colesteryl myristate;

Total active products present: 17.

Extraction yield: 80% out of a total of 85-90% of active substances.

A propolis solution is then obtained, by means of the method of the present invention, in which terpenes, ter-

penoids, flavonoids and steroids are present as free molecules.

In particular, the propolis solution analyzed above, refers to a particular type of propolis produced in Northern Italy, more precisely in the Asiago/Lavorano-Folgaria plateau. It is known that propolis has a composition which varies according to the type of flora of the area where the beehives are positioned.

Miscibility of the solutions.

The solutions extracted prove to be miscible with each other in all proportions.

Miscibility with essential oils.

The solutions extracted prove to be miscible with all types of essential oils in any proportion with each other.

Miscibility with water.

The solutions extracted prove to be miscible with water up to a maximum of 25% of distilled water whatever the proportion of the solutions with each other.

Evaporation and aero-dispersion

The solutions extracted are completely aero-dispersible within a temperature range of 40°C to 90°C with the use of thermo-emanators or electro-emanators as described in Italian patent Nr. 1287235.

Concentration

The extraction method according to the present invention also allows solutions to be obtained at different concentrations.

By varying, in fact, the ratio between time/temperature/magnetic field intensity, it is possible to cover the whole range of extractions of all types of resins and/or essential oils partially soluble in:

- Ethanol (CH_3CH_2OH)
- Ethyl ethanoate ($CH_3CO_2CH_2$)

and mixtures of these two solvents in any proportion with each other.

This characteristic makes the extraction method according to the present invention extremely versatile and elastic and allows the extraction and preparation of alcohol and/or hydro-alcohol solutions containing the active principles in different concentrations according to the end-use of the product.

Specific characteristics of the alcohol and/or hydro-alcohol solutions obtained with the extraction method according to the present invention are the following.

Contrary to normal essential oils extracted in a vapour stream, or with supercritical CO_2 and normal alcohol and/or hydro-alcohol solutions obtained from natural resins, the solutions extracted from natural resins and/or essential oils, produced with the method according to the

present invention, allow not only monoterpenes and some sesquiterpenes, but also all (C_{15}) sesquiterpenes, (C_{20}) diterpenes and (C_{30}) triterpenes, flavonoids and steroids, to be dispersible in air.

This is possible because the single solute molecular structures are free molecules. The magnetic field, in fact, releases the molecular structures of the terpenes and/or terpenoids from the polymeric part of the resins made up of starches, rubber based on isoprene and rosinic resin (rosin).

Analogously, in the case of essential oils, the process according to the present invention allows solutions of essential oils to be obtained, in which the single solute molecular structures, terpenes and/or terpenoids, are free molecules.

This was not possible so far with the known extraction systems including systems using N_2 and H_2O in supercritical form.

The essential oils and mixtures of compounds extracted from resins in a vapour stream or with supercritical gases are not made up of free molecules and when they are heated to temperatures exceeding 50°C, they interact forming new molecular structures, in particular compounds of the rubber type (isoprene, rosin) which cross-link forming a solid polymeric structure which is

therefore absolutely non-aero-dispersible.

The method according to the present invention forms an absolute novelty in the field of terpene and/or terpenoid extraction from natural resins such as incense, myrrh, dacryodes, dammar, propolis.

The method according to the present invention very surprisingly also allows triterpenes such as boswellic acids and commiphoric acids, having the formula $C_{30}H_{48}O_3$, $C_{30}H_{48}O_5$, $C_{30}H_{50}O_3$, to be aero-dispersed in a controlled quantity and time.

Aero-dispersion of the solutions.

The method according to the present invention very surprisingly allows solutions to be obtained, containing aero-dispersible compounds with a vast rang of molecular weights from MW 136 (monoterpenes) to MW 532 (pentacyclic triterpenes), and with a molecular weight higher than 600 dalton.

It is therefore possible to contemporaneously disperse in air terpenes and/or terpenoids with a different molecular weight, which varies within the range of MW 136 to MW 532.

This means that all terpenes and/or terpenoids contained in natural resins or in essential oils can be dispersed in the air in a different time depending on the temperature.

In practice, the dispersion time at 50°C proves to be 240 minutes, whereas at 90°C it is 60 minutes.

In particular the following compositions are described, obtained by mixing the fractions of terpenes and/or terpenoids extracted by means of the method according to the present invention.

Composition nr. 1

A composition is described, obtained by mixing the following components in the amounts indicated with respect to 1 ml of composition. Incense and myrrh are the terpene and/or terpenoid fractions extracted from incense and myrrh according to the method of the present invention, as described in example 1, whereas Hyssopus officinalis decumbens consists of the fractions extracted according to the method of the present invention from the essential oil Hyssopus officinalis decumbens.

Hyssopus officinalis decumbens	0.0271 ml;
green tangerine	0.0075 ml;
myrrh	0.125 ml;
incense	0.125 ml;
ethanol	0.5854 ml;
distilled water	0.130 ml.

Composition nr. 2

A composition is described, obtained by mixing the following components in the amounts indicated with respect

to 1 ml of composition. Incense and myrrh are the terpene and/or terpenoid fractions extracted from incense and myrrh according to the method of the present invention, as described in example 1, whereas Hyssopus officinalis aristatus consists of the fractions extracted according to the method of the present invention from the essential oil Hyssopus officinalis aristatus.

Hyssopus officinalis aristatus	0.0271 ml;
green tangerine	0.0075 ml;
myrrh	0.125 ml;
incense	0.125 ml;
ethanol	0.5954 ml;
distilled water	0.120 ml.

Composition nr. 3

A composition is described, obtained by mixing composition nr. 1 and the fractions of terpenes and/or terpenoids extracted from propolis as described in example 2. The values are volume percentages.

Composition nr. 1	80%;
propolis	20%.

Composition nr. 4

A composition is described, obtained by mixing composition nr. 2 with the fractions of terpenes and terpenoids extracted from propolis as described in example 2. The values are volume percentages.

Composition nr. 2	80%;
propolis	20%.

In particular, the compositions according to the present invention can be used as aero-dispersible solutions and as solutions for topic use.